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I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Mail Stop Petition, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: February 5, 2004

Signature: 

Eric M. Brusca

\$ DAC

Docket No.: 27013/38150
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

FEB 12 2004

OFFICE OF PETITIONS

Re Patent Application of:
Lutz Gissman and Martin Mueller

Application No.: 10/042,526

Group Art Unit: 1648 ✓

Filed: January 8, 2002

Examiner: Not Assigned

For: Papilloma Virus Capsomere Vaccine
Formulations and Methods of Use

**PETITION TO WITHDRAWAL HOLDING OF ABANDONMENT
PURSUANT TO 37 C.F.R. 1.181(A)**

MS Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This petition is being filed pursuant to 37 C.F.R. 1.181(a) to request withdrawal of a holding of abandonment for asserted failure to reply to a Notice to File Missing Parts mailed on February 13, 2002.

Applicants submit that a response (a copy of which is attached as **Exhibit A**) to the Notice to File Missing Parts of Nonprovisional Application was filed on April 15, 2002. The response included the fee for a one month extension of time pursuant to 37 CFR 1.17(a) as indicated on a Fee Transmittal form (a copy of which is attached as **Exhibit B**) which was filed concurrently with the response. Even though the Applicants did not include a petition for the extension of time, 37 CFR 1.136(a)(3) states that "submission of the fee set forth in 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." The response was therefore timely filed. Also attached is a date-stamped post card (attached as **Exhibit C**) returned from the Patent Office showing that the response and the fee were received on April 29, 2002.

Adjustment date: 05/05/2004 SDIRETA1
02/10/2004 SDESHE1 00000026 10042526
01 FC:1480 -130.00 OP

Reply/Ref: 05/05/2004 SDIRETA1 0013321500
Date: 02/06/04 Name/Number: 10042526
FC: 3504 \$130.00 CR

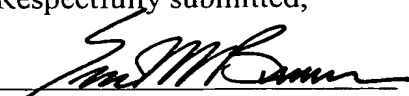
Attached herewith is a check in the amount of \$130.00 to cover the basic petition filing fee pursuant to 37 CFR §1.17(h). However, Applicants request that any over-payment be refunded to deposit account number 13-2855.

In light of the foregoing, Applicants respectfully request that this petition to withdrawal the holding of abandonment be granted.

Dated: February 5, 2004

Respectfully submitted,

By


Eric M. Brusca

Registration No.: 52,664

MARSHALL, GERSTEIN & BORUN LLP

233 S. Wacker Drive

6300 Sears Tower

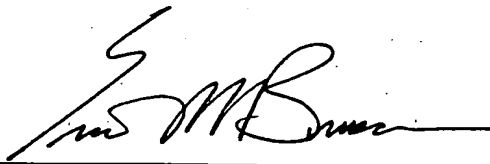
Chicago, Illinois 60606-6357

(312) 474-6300

Attorneys for Applicant

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Lutz Gissman and Martin Mueller)	I hereby certify that this paper is being
)	deposited with the United States Postal
Application Serial No. 10/042,526)	Service as First Class mail, postage
Filed: January 8, 2002)	prepaid, in an envelope addressed to:
)	Commissioner for Patents, Washington,
For: Papilloma Virus Capsomere)	D.C. 20231, on April 15, 2002.
Vaccine Formulations and Methods of Use)	
)	
Group Art Unit: 1648)	
)	
Examiner: TBD)	



Eric M. Brusca

**APPLICANTS' RESPONSE TO NOTICE TO FILE MISSING PARTS OF
NONPROVISIONAL APPLICATION**

Commissioner for Patents
Washington, DC 20231

Sir:

In a Notice to File Missing Parts mailed February 2, 2002, various items were found to be missing in the above-identified application.

REMARKS

In order to avoid abandonment of the instant application, the Applicants file herewith a check covering the statutory basic filing fee and a late filing fee of \$130 as set forth in 37 CFR 1.16(l).

Additionally, a substitute specification in compliance with 37 CFR 1.52 is submitted herewith. The Applicants would like to point out that pages 1 and 2 of the specification have been corrected.

A copy of the Sequence Listing in computer readable form and corresponding statement that the content of the Sequence Listing information recorded in computer readable form is identical to the written Sequence Listing has been previously filed in the related application U.S.S.N 09/632,286. Thus, the Applicants submit that in accordance with 37 CFR 1.821(e), the sequence listing in the instant application is identical to that of U.S.S.N 09/632,286. The Applicants request that the computer-readable sequence listing in U.S.S.N 09/632,286 be entered in the present application.

AMENDMENTS

In the specification:

Please replace the specification as originally filed with the substitute specification submitted herewith (attached hereto as **Appendix A**). A marked-up copy of the substitute specification appears in **Appendix B** which indicates where the changes have been made. The Applicants hereby state that the substitute specification includes no new matter.

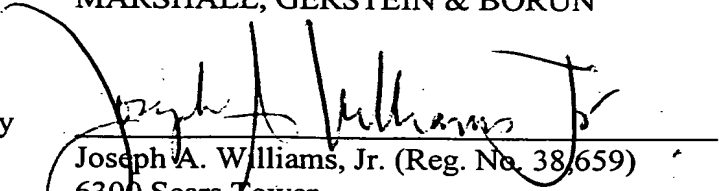
SUMMARY

In view of the amendments and remarks made herein, the Applicants believes that the application is formal and in good standing.

Respectfully submitted,

MARSHALL, GERSTEIN & BORUN

By



Joseph A. Williams, Jr. (Reg. No. 38,659)
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

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PAPILLOMA VIRUS CAPSOMERE VACCINE FORMULATIONS AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to vaccine formulations
5 comprising papilloma virus proteins, either as fusion proteins, truncated
proteins, or truncated fusion proteins. The invention further embraces
methods for producing capsomeres of the formulations, as well as
prophylactic and therapeutic methods for their use.

BACKGROUND

10 Infections with certain high-risk strains of genital papilloma
viruses in humans (HPV) -- for example, HPV 16, 18, or 45 -- are
believed to be the main risk factor for the formation of malignant tumors of
the anogenital tract. Of the possible malignancies, cervical carcinoma is by
far the most frequent; according to an estimate by the World Health
15 Organization (WHO), almost 500,000 new cases of the disease occur
annually. Because of the frequency with which this pathology occurs, the
connection between HPV infection and cervical carcinoma has been
extensively examined, leading to numerous generalizations.

For example, precursor lesions of cervical intraepithelial
20 neoplasia (CIN) are known to be caused by papilloma virus infections
[Crum, *New Eng. J. Med.* 310:880-883 (1984)]. DNA from the genomes
of certain HPV types, including for example, strains 16, 18, 33, 35, and
45, have been detected in more than 95% of tumor biopsies from patients
with this disorder, as well as in primary cell lines cultured from the
25 tumors. Approximately 50 to 70% of the biopsied CIN tumor cells have
been found to include DNA derived only from HPV 16.

The protein products of the HPV 16 and HPV 18 early genes
E6 and E7 have been detected in cervical carcinoma cell lines as well as in

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human keratinocytes transformed *in vitro* [Wettstein, *et al.*, in PAPILLOMA
VIRUSES AND HUMAN CANCER, Pfister (Ed.), CRC Press: Boca Raton, FL
1990 pp 155-179] and a significant percentage of patients with cervical
carcinoma have anti-E6 or anti-E7 antibodies. The E6 and E7 proteins
5 have been shown to participate in induction of cellular DNA synthesis in
human cells, transformation of human keratinocytes and other cell types,
and tumor formation in transgenic mice [Arbelt, *et al.*, *J. Virol.*, 68:4358-
4364 (1994); Auewarakul, *et al.*, *Mol. Cell. Biol.* 14:8250-8258 (1994);
Barbosa, *et al.*, *J. Virol.* 65:292-298 (1991); Kaur, *et al.*, *J. Gen. Virol.*
10 70:1261-1266 (1989); Schlegel, *et al.*, *EMBO J.*, 7:3181-3187 (1988)].
The constitutive expression of the E6/E7 proteins appears to be necessary
to maintain the transformed condition of HPV-positive tumors.

Despite the capacity of some HPV strains to induce
neoplastic phenotypes *in vivo* and *in vitro*, still other HPV types cause
15 benign genital warts such as condylomata acuminata and are only rarely
associated with malignant tumors [Ikenberg. In Gross, *et al.*, (eds.)
GENITAL PAPILLOMAVIRUS INFECTIONS. Springer Verlag: Berlin, pp., 87-
112]. Low risk strains of this type include, for example, HPV 6 and 11.

Most often, genital papilloma viruses are transmitted between
20 humans during intercourse which in many instances leads to persistent
infection in the anogenital mucous membrane. While this observation
suggests that either the primary infection induces an inadequate immune
response or that the virus has developed the ability to avoid immune
surveillance, other observations suggest that the immune system is active
25 during primary manifestation as well as during malignant progression of
papilloma virus infections [Altmann *et al.* in VIRUSES AND CANCER,
Minson *et al.*, (eds.) Cambridge University Press, (1994) pp. 71-80].

For example, the clinical manifestation of primary infection
by rabbit and bovine papilloma virus can be prevented by vaccination with
30 wart extracts or viral structural proteins [Altmann, *et al.*, *supra*; Campo,

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<h1 style="margin: 0;">FEE TRANSMITTAL</h1> <h2 style="margin: 0;">for FY 2002</h2> <p style="margin: 0; font-size: small;">Patent fees are subject to annual revision.</p>		Complete if Known	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27		Application Number	
		Filing Date	
		First Named Inventor	
		Examiner Name	
		Group Art Unit	
TOTAL AMOUNT OF PAYMENT (\$)		1,314.00	
		Attorney Docket No.	
		27013/38150	

METHOD OF PAYMENT (check all that apply) <input checked="" type="checkbox"/> Check <input type="checkbox"/> Credit Card <input type="checkbox"/> Money Order <input type="checkbox"/> Other <input type="checkbox"/> None <input type="checkbox"/> Deposit Account Deposit Account Number: 13-2855 Deposit Account Name: Marshall, Gerstein & Borun The Commissioner is hereby authorized to: (check all that apply) <input type="checkbox"/> Charge fee(s) indicated below <input type="checkbox"/> Credit any overpayments <input checked="" type="checkbox"/> Charge any additional fee(s) during the pendency of this application <input type="checkbox"/> Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.		FEE CALCULATION (continued)																																																																																																																																																																																																																																													
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SUBMITTED BY		Complete (if applicable)	
Name (Print/Type)	Joseph A. Williams, Jr.	Registration No. (Attorney/Agent)	38,659
Signature		Telephone	312.474-6300
		Date	April 15, 2002

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail, in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on the date shown below.	
Dated: April 15, 2002	Signature: (Name of Signer) Joseph A. Williams, Jr.

27013/38150

4/15/02 /16

The Patent Office is hereby requested to acknowledge receipt

of the following ~~parts~~ ^{parts} of dumping and returning this card.

Cleaman et al.

10/042,536

Amendment transmits 1,314.00 - ck# 71295

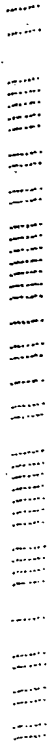
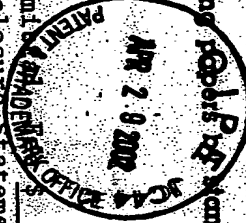
Information Disclosure Statement w/form PTO-1449;

Applicants' response to notice to file missing parts of

nonprovisional application; Appendix A - substitute spec.,

Appendix B - marked up copy of spec.,

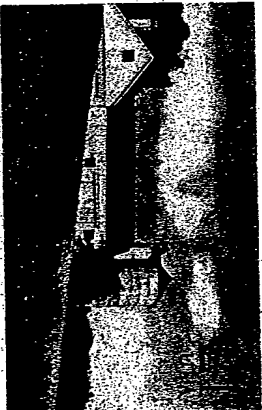
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MARSHALL GERSTEIN

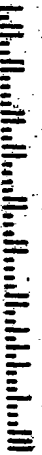


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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/042,526	01/08/2002	Lutz Gissman	27013/38150

CONFIRMATION NO. 9119

ABANDONMENT/TERMINATION
LETTER

04743
 MARSHALL, GERSTEIN & BORUN LLP
 6300 SEARS TOWER
 233 S. WACKER DRIVE
 CHICAGO, IL 60606

RECEIVED



OC000000011719082

JAN 28 2004

MARSHALL GERSTEIN

Date Mailed: 01/20/2004

NOTICE OF ABANDONMENT UNDER 37 CFR 1.53 (f) OR (g)

The above-identified application is abandoned for failure to timely or properly reply to the Notice to File Missing Parts (Notice) mailed on 02/13/2002.

- No reply was received.

A petition to the Commissioner under 37 CFR 1.137 may be filed requesting that the application be revived.

Under 37 CFR 1.137(a), a petition requesting the application be revived on the grounds of **UNAVOIDABLE DELAY** must be filed promptly after the applicant becomes aware of the abandonment and such petition must be accompanied by: (1) an adequate showing of the cause of unavoidable delay; (2) the required reply to the above-identified Notice; (3) the petition fee set forth in 37 CFR 1.17(l); and (4) a terminal disclaimer if required by 37 CFR 1.137(d).

Under 37 CFR 1.137(b), a petition requesting the application be revived on the grounds of **UNINTENTIONAL DELAY** must be filed promptly after applicant becomes aware of the abandonment and such petition must be accompanied by: (1) a statement that the entire delay was unintentional; (2) the required reply to the above-identified Notice; (3) the petition fee set forth in 37 CFR 1.17(m); and (4) a terminal disclaimer if required by 37 CFR 1.137(d).

Any questions concerning petitions to revive should be directed to the "Office of Petitions" at (703) 305-9282. Petitions should be mailed to: Mail Stop Petitions, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

*A copy of this notice **MUST** be returned with the reply.*

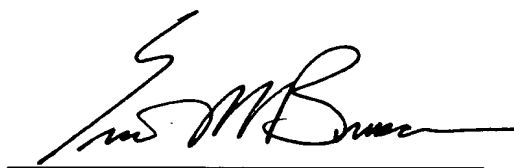
Customer Service Center

Initial Patent Examination Division (703) 308-1202

PART 1 - ATTORNEY/APPLICANT COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Lutz Gissman and Martin Mueller)	I hereby certify that this paper is being
)	deposited with the United States Postal
Application Serial No. 10/042,526)	Service as First Class mail, postage
)	prepaid, in an envelope addressed to:
Filed: January 8, 2002)	Commissioner for Patents, Washington,
)	D.C. 20231, on April 15, 2002.
For: Papilloma Virus Capsomere)	
Vaccine Formulations and Methods of)	
Use)	
)	
Group Art Unit: 1648)	
)	
Examiner: TBD)	



Eric M. Brusca

**APPLICANTS' RESPONSE TO NOTICE TO FILE MISSING PARTS OF
NONPROVISIONAL APPLICATION**

Commissioner for Patents
Washington, DC 20231

Sir:

In a Notice to File Missing Parts mailed February 2, 2002, various items were found to be missing in the above-identified application.

REMARKS

In order to avoid abandonment of the instant application, the Applicants file herewith a check covering the statutory basic filing fee and a late filing fee of \$130 as set forth in 37 CFR 1.16(l).

Additionally, a substitute specification in compliance with 37 CFR 1.52 is submitted herewith. The Applicants would like to point out that pages 1 and 2 of the specification have been corrected.

A copy of the Sequence Listing in computer readable form and corresponding statement that the content of the Sequence Listing information recorded in computer readable form is identical to the written Sequence Listing has been previously filed in the related application U.S.S.N 09/632,286. Thus, the Applicants submit that in accordance with 37 CFR 1.821(e), the sequence listing in the instant application is identical to that of U.S.S.N 09/632,286. The Applicants request that the computer-readable sequence listing in U.S.S.N 09/632,286 be entered in the present application.

AMENDMENTS

In the specification:

Please replace the specification as originally filed with the substitute specification submitted herewith (attached hereto as **Appendix A**). A marked-up copy of the substitute specification appears in **Appendix B** which indicates where the changes have been made. The Applicants hereby state that the substitute specification includes no new matter.

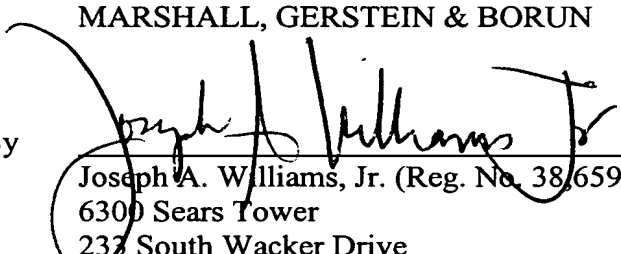
SUMMARY

In view of the amendments and remarks made herein, the Applicants believes that the application is formal and in good standing.

Respectfully submitted,

MARSHALL, GERSTEIN & BORUN

By



Joseph A. Williams, Jr. (Reg. No. 38,659)
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APPENDIX A

PAPILLOMA VIRUS CAPSOMERE VACCINE FORMULATIONS AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to vaccine formulations
5 comprising papilloma virus proteins, either as fusion proteins, truncated
proteins, or truncated fusion proteins. The invention further embraces
methods for producing capsomeres of the formulations, as well as
prophylactic and therapeutic methods for their use.

BACKGROUND

10 Infections with certain high-risk strains of genital papilloma
viruses in humans (HPV) -- for example, HPV 16, 18, or 45 -- are
believed to be the main risk factor for the formation of malignant tumors of
the anogenital tract. Of the possible malignancies, cervical carcinoma is by
far the most frequent: according to an estimate by the World Health
15 Organization (WHO), almost 500,000 new cases of the disease occur
annually. Because of the frequency with which this pathology occurs, the
connection between HPV infection and cervical carcinoma has been
extensively examined, leading to numerous generalizations.

For example, precursor lesions of cervical intraepithelial
20 neoplasia (CIN) are known to be caused by papilloma virus infections
[Crum. *New Eng. J. Med.* 310:880-883 (1984)]. DNA from the genomes
of certain HPV types, including for example, strains 16, 18, 33, 35, and
45, have been detected in more than 95% of tumor biopsies from patients
with this disorder, as well as in primary cell lines cultured from the
25 tumors. Approximately 50 to 70% of the biopsied CIN tumor cells have
been found to include DNA derived only from HPV 16.

The protein products of the HPV 16 and HPV 18 early genes
E6 and E7 have been detected in cervical carcinoma cell lines as well as in

human keratinocytes transformed *in vitro* [Wettstein, *et al.*, in PAPILLOMA
VIRUSES AND HUMAN CANCER, Pfister (Ed.), CRC Press: Boca Raton, FL
1990 pp 155-179] and a significant percentage of patients with cervical
carcinoma have anti-E6 or anti-E7 antibodies. The E6 and E7 proteins
5 have been shown to participate in induction of cellular DNA synthesis in
human cells, transformation of human keratinocytes and other cell types,
and tumor formation in transgenic mice [Arbelt, *et al.*, *J. Virol.*, 68:4358-
4364 (1994); Auewarakul, *et al.*, *Mol. Cell. Biol.* 14:8250-8258 (1994);
Barbosa, *et al.*, *J. Virol.* 65:292-298 (1991); Kaur, *et al.*, *J. Gen. Virol.*
10 70:1261-1266 (1989); Schlegel, *et al.*, *EMBO J.*, 7:3181-3187 (1988)].
The constitutive expression of the E6/E7 proteins appears to be necessary
to maintain the transformed condition of HPV-positive tumors.

Despite the capacity of some HPV strains to induce
neoplastic phenotypes *in vivo* and *in vitro*, still other HPV types cause
15 benign genital warts such as condylomata acuminata and are only rarely
associated with malignant tumors [Ikenberg. In Gross, *et al.*, (eds.)
GENITAL PAPILLOMAVIRUS INFECTIONS. Springer Verlag: Berlin, pp., 87-
112]. Low risk strains of this type include, for example, HPV 6 and 11.

Most often, genital papilloma viruses are transmitted between
20 humans during intercourse which in many instances leads to persistent
infection in the anogenital mucous membrane. While this observation
suggests that either the primary infection induces an inadequate immune
response or that the virus has developed the ability to avoid immune
surveillance, other observations suggest that the immune system is active
25 during primary manifestation as well as during malignant progression of
papilloma virus infections [Altmann *et al.* in VIRUSES AND CANCER,
Minson *et al.*, (eds.) Cambridge University Press, (1994) pp. 71-80].

For example, the clinical manifestation of primary infection
by rabbit and bovine papilloma virus can be prevented by vaccination with
30 wart extracts or viral structural proteins [Altmann, *et al.*, *supra*; Campo,

Curr. Top. In Microbiol and Immunol. 186:255-266 (1994); Yindle and Frazer, Curr. Top. In Microbiol. and Immunol. 186:217-253 (1994)].

Rodents previously vaccinated with vaccinia recombinants encoding HPV 16 early proteins E6 or E7, or with synthetic E6 or E7 peptides, are
5 similarly protected from tumor formation after inoculation of HPV 16 transformed autologous cells [Altman, *et al.*, *supra*; Campo, *et al.*, *supra*; Yindle and Frazer, *et al. supra*]. Regression of warts can be induced by the transfer of lymphocytes from regressor animals following infection by animal papilloma viruses. Finally, in immunosuppressed patients, such as,
10 for example, recipients of organ transplants or individuals infected with HIV, the incidence of genital warts, CIN, and anogenital cancer is elevated.

To date, no HPV vaccinations have been described which comprise human papilloma virus late L1 protein in the form of capsomeres
15 which are suitable both for prophylactic and therapeutic purposes. Since the L1 protein is not present in malignant genital lesions, vaccination with L1 protein does not have any therapeutic potential for these patients. Construction of chimeric proteins, comprising amino acid residues from L1 protein and, for example E6 or E7 protein, which give rise to chimeric
20 capsomeres, combines prophylactic and therapeutic functions of a vaccine. A method for high level production of chimeric capsomeres would therefore be particularly desirable, in view of the possible advantages offered by such a vaccine for prophylactic and therapeutic intervention.

Thus there exists a need in the art to provide vaccine
25 formulations which can prevent or treat HPV infection. Methods to produce vaccine formulations which overcome problems known in the art to be associated with recombinant HPV protein expression and purification would manifestly be useful to treat the population of individuals already infected with HPV as well as useful to immunize the population of
30 individuals susceptible to HPV infection.

SUMMARY OF THE INVENTION

The present invention provides therapeutic and prophylactic vaccine formulations comprising chimeric human papilloma capsomeres.

The invention also provides therapeutic methods for treating patients

5 infected with an HPV as well as prophylactic methods for preventing HPV infection in a susceptible individual. Methods for production and purification of capsomeres and proteins of the invention are also contemplated.

In one aspect of the invention, prophylactic vaccinations for
10 prevention of HPV infection are considered which incorporate the structural proteins L1 and L2 of the papilloma virus. Development of a vaccine of this type faces significant obstacles because papilloma viruses cannot be propagated to adequate titers in cell cultures or other experimental systems to provide the viral proteins in sufficient quantity for economical vaccine
15 production. Moreover, recombinant methodologies to express the proteins are not always straightforward and often results in low protein yield. Recently, virus-like particles (VLPs), similar in make up to viral capsid structures, have been described which are formed in Sf-9 insect cells upon expression of the viral proteins L1 and L2 (or L1 on its own) using
20 recombinant vaccinia or baculovirus. Purification of the VLPs can be achieved very simply by means of centrifugation in CsCl or sucrose gradients [Kimbauer, *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 99:12180-12814 (1992); Kimbauer, *et al.*, *J. Virol.* 67:6929-6936 (1994); Proso, *et al.*, *J. Virol.* 67:14:1936-1944 (1992); Sasagawa, *et al.*, *Virology* 201:126-195 (1995); Volpers, *et al.*, *J. Virol.* 69:3258-3264 (1995); Zhou, *et al.*, *J. Gen. Virol.* 74:762-769 (1993); Zhou, *et al.*, *Virology* 185:251-257 (1991)]. WO 93/02184 describes a method in which papilloma virus-like particles (VLPs) are used for diagnostic applications or as a vaccine against
25 infections caused by the papilloma virus. WO 94/00152 describes

recombinant production of L1 protein which mimics the conformational neutralizing epitope on human and animal papilloma virions.

In another aspect of the invention, therapeutic vaccinations are provided to relieve complications of, for example, cervical carcinoma or precursor lesions resulting from papilloma virus infection, and thus represent an alternative to prophylactic intervention. Vaccinations of this type may comprise early papilloma virus proteins, principally E6 or E7, which are expressed in the persistently infected cells. It is assumed that, following administration of a vaccination of this type, cytotoxic T-cells might be activated against persistently infected cells in genital lesions. The target population for therapeutic intervention is patients with HPV-associated pre-malignant or malignant genital lesions. PCT patent application WO 93/20844 discloses that the early protein E7 and antigenic fragments thereof of the papilloma virus from HPV or BPV is therapeutically effective in the regression, but not in the prevention, of papilloma virus tumors in mammals. While early HPV proteins have been produced by recombinant expression in *E. coli* or suitable eukaryotic cell types, purification of the recombinant proteins has proven difficult due to inherent low solubility and complex purification procedures which generally require a combination of steps, including ion exchange chromatography, gel filtration and affinity chromatography.

According to the present invention, vaccine formulations comprising papilloma virus capsomeres are provided which comprise either: (i) a first protein that is an intact viral protein expressed as a fusion protein comprised in part of amino acid residues from a second protein; (ii) a truncated viral protein; (iii) a truncated viral protein expressed as a fusion protein comprised in part of amino acid residues from a second protein, or (iv) some combination of the three types of proteins. According to the invention, vaccine formulations are provided comprising capsomeres of bovine papilloma virus (BPV) and human papilloma virus. Preferred

bovine virus capsomeres comprise protein from bovine papilloma virus type

I. Preferred human virus capsomeres comprise proteins from any one of human papilloma virus strains HPV6, HPV11, HPV16, HPV18, HPV33, HPV35, and HPV45. The most preferred vaccine formulations comprise capsomeres comprising proteins from HPV16.

In one aspect, capsomere vaccine formulations of the invention comprise a first intact viral protein expressed as a fusion protein with additional amino acid residues from a second protein. Preferred intact viral proteins are the structural papilloma viral proteins L1 and L2.

Capsomeres comprised of intact viral protein fusions may be produced using the L1 and L2 proteins together or the L1 protein alone. Preferred capsomeres are made up entirely of L1 fusion proteins, the amino acid sequence of which is set out in SEQ ID NO: 2 and encoded by the polynucleotide sequence of SEQ ID NO: 1. Amino acids of the second protein can be derived from numerous sources (including amino acid residues from the first protein) as long as the addition of the second protein amino acid residues to the first protein permits formation of capsomeres. Preferably, addition of the second protein amino acid residues inhibits the ability of the intact viral protein to form virus-like particle structures; most preferably, the second protein amino acid residues promote capsomere formation. In one embodiment of the invention, the second protein may be any human tumor antigen, viral antigen, or bacterial antigen which is important in stimulating an immune response in neoplastic or infectious disease states. In a preferred embodiment, the second protein is also a papilloma virus protein. It also preferred that the second protein be the expression product of papilloma virus early gene. It is also preferred, however, that the second protein be selected from group of E1, E2, E3, E4, E5, E6, and E7 -- early gene products encoded in the genome of papilloma virus strains HPV6, HPV11, HPV18, HPV33, HPV35, or HPV45. It is most preferred that the second protein be encoded by the HPV16

E7 gene, the open reading frame of which is set out in SEQ ID NO: 3. Capsomeres assembled from fusion protein subunits are referred to herein as chimeric capsomeres. In one embodiment, the vaccine formulation of the invention is comprised of chimeric capsomeres wherein L1 protein
5 amino acid residues make up approximately 50 to 99% of the total fusion protein amino acid residues. In another embodiment, L1 amino acid residues make up approximately 60 to 90% of the total fusion protein amino acid residues; in a particularly preferred embodiment, L1 amino acids comprise approximately 80% of the fusion protein amino acid
10 residues.

In another aspect of the invention, capsomere vaccine formulations are provided that are comprised of truncated viral proteins having a deletion of one or more amino acid residues necessary for formation of a virus-like particle. It is preferred that the amino acid
15 deletion not inhibit formation of capsomeres by the truncated protein, and it is most preferred that the deletion favor capsomere formation. Preferred vaccine formulations of this type include capsomeres comprised of truncated L1 with or without L2 viral proteins. Particularly preferred capsomeres are comprised of truncated L1 proteins. Truncated proteins
20 contemplated by the invention include those having one or more amino acid residues deleted from the carboxy terminus of the protein, or one or more amino acid residues deleted from the amino terminus of the protein, or one or more amino acid residues deleted from an internal region (*i.e.*, not from either terminus) of the protein. Preferred capsomere vaccine formulations
25 are comprised of proteins truncated at the carboxy terminus. In formulations including L1 protein derived from HPV16, it is preferred that from 1 to 34 carboxy terminal amino acid residues are deleted. Relatively shorter deletions are also contemplated which offer the advantage of minor modification of the antigenic properties of the L1 proteins and the
30 capsomeres formed thereof. It is most preferred, however, that 34 amino

acid residues be deleted from the L1 sequence, corresponding to amino acids 472 to 505 in HPV16 set out in SEQ ID NO: 2, and encoded by the polynucleotide sequence corresponding to nucleotides 1414 to 1516 in the human HPV16 L1 coding sequence set out in SEQ ID NO: 1.

5 When a capsomere vaccine formulation is made up of proteins bearing an internal deletion, it is preferred that the deleted amino acid sequence comprise the nuclear localization region of the protein. In the L1 protein of HPV 16, the nuclear localization signal is found from about amino acid residue 499 to about amino acid residue 505. Following
10 expression of L1 proteins wherein the NLS has been deleted, assembly of capsomere structures occurs in the cytoplasm of the host cell. Consequently, purification of the capsomeres is possible from the cytoplasm instead of from the nucleus where intact L1 proteins assemble into capsomeres. Capsomeres which result from assembly of truncated
15 proteins wherein additional amino acid sequences do not replace the deleted protein sequences are necessarily not chimeric in nature.

 In still another aspect of the invention, capsomere vaccine formulations are provided comprising truncated viral protein expressed as a fusion protein adjacent amino acid residues from a second protein.
20 Preferred truncated viral proteins of the invention are the structural papilloma viral proteins L1 and L2. Capsomeres comprised of truncated viral protein fusions may be produced using L1 and L2 protein components together or L1 protein alone. Preferred capsomeres are those comprised of L1 protein amino acid residues. Truncated viral protein components of the
25 fusion proteins include those having one or more amino acid residues deleted from the carboxy terminus of the protein, or one or more amino acid residues deleted from the amino terminus of the protein, or one or more amino acid residues deleted from an internal region (*i.e.*, not from either terminus) of the protein. Preferred capsomere vaccine formulations
30 are comprised of proteins truncated at the carboxy terminus. In those

formulations including L1 protein derived from HPV16, it is preferred that from 1 to 34 carboxy terminal amino acid residues are deleted. Relatively shorter deletions are also contemplated that offer the advantage of minor modification of the antigenic properties of the L1 protein component of the fusion protein and the capsomeres formed thereof. It is most preferred, however, that 34 amino acid residues be deleted from the L1 sequence, corresponding to amino acids 472 to 505 in HPV16 set out in SEQ ID NO: 2, and encoded by the polynucleotide sequence corresponding to nucleotides 1414 to 1516 in the human HPV16 L1 coding sequence set out in SEQ ID NO: 1. When the vaccine formulation is comprised of capsomeres made up of proteins bearing an internal deletion, it is preferred that the deleted amino acid sequence comprise the nuclear localization region, or sequence, of the protein.

Amino acids of the second protein can be derived from numerous sources as long as the addition of the second protein amino acid residues to the first protein permits formation of capsomeres. Preferably, addition of the second protein amino acid residues promotes or favors capsomere formation. Amino acid residues of the second protein can be derived from numerous sources, including amino acid residues from the first protein. In a preferred embodiment, the second protein is also a papilloma virus protein. It is also preferred that the second protein be the expression product of papilloma virus early gene. It is most preferred, however, that the second protein be selected from group of early gene products encoding by papilloma virus E1, E2, E3, E4, E5, E6, and E7 genes. In one embodiment, the vaccine formulation of the invention is comprised of chimeric capsomeres wherein L1 protein amino acid residues make up approximately 50 to 99% of the total fusion protein amino acid residues. In another embodiment, L1 amino acid residues make up approximately 60 to 90% of the total fusion protein amino acid residues; in

a particularly preferred embodiment, L1 amino acids comprise approximately 80% of the fusion protein amino acid residues.

In a preferred embodiment of the invention, proteins of the vaccine formulations are produced by recombinant methodologies, but in formulations comprising intact viral protein, the proteins may be isolated from natural sources. Intact proteins isolated from natural sources may be modified *in vitro* to include additional amino acid residues to provide a fusion protein of the invention using covalent modification techniques well known and routinely practiced in the art. Similarly, in formulations comprising truncated viral proteins, the proteins may be isolated from natural sources as intact proteins and hydrolyzed *in vitro* using chemical hydrolysis or enzymatic digestion with any of a number of site-specific or general proteases, the truncated protein subsequently modified to include additional amino acid residues as described above to provide a truncated fusion protein of the invention.

In producing capsomeres, recombinant molecular biology techniques can be utilized to produce DNA encoding either the desired intact protein, the truncated protein, or the truncated fusion protein. Recombinant methodologies required to produce a DNA encoding a desired protein are well known and routinely practiced in the art. Laboratory manuals, for example Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Press: Cold Spring Harbor, NY (1989) and Ausubel *et al.*, (eds.), PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. (1994-1997), describe in detail techniques necessary to carry out the required DNA manipulations. For large-scale production of chimeric capsomeres, protein expression can be carried out using either viral or eukaryotic vectors. Preferable vectors include any of the well known prokaryotic expression vectors, recombinant baculoviruses, COS cell specific vectors, vaccinia recombinants, or yeast-specific expression constructs. When recombinant proteins are used to

provide capsomeres of the invention, the proteins may first be isolated from the host cell of its expression and thereafter incubated under conditions which permit self-assembly to provide capsomeres. Alternatively, the proteins may be expressed under conditions wherein capsomeres are formed
5 in the host cell.

The invention also contemplates processes for producing capsomeres of the vaccine formulations. In one method, L1 proteins are expressed from DNA encoding six additional histidines at the carboxy terminus of the L1 protein coding sequence. L1 proteins expressed with
10 additional histidines (His L1 proteins) are most preferably expressed in *E. coli* and the His L1 proteins can be purified using nickel affinity chromatography. His L1 proteins in cell lysate are suspended in a denaturation buffer, for example, 6 M guanidine hydrochloride or a buffer of equivalent denaturing capacity, and then subjected to nickel
15 chromatography. Protein eluted from the nickel chromatography step is renatured, for example in 150 mM NaCl, 1 mM CaCl_2 , 0.01 % Triton-X 100, 10 mM HEPES (N-2-hydroxyethyl piperazine-N'-2 ethane sulfonic acid), pH 7.4. According to a preferred method of the invention, assembly of capsomeres takes place after dialysis of the purified proteins, preferably
20 after dialysis against 150 mM NaCl, 25 mM Ca^{2+} , 10 % DMSO (dimethyl sulfoxide), 0.1 % Triton-X 100, 10 mM Tris [tris-(hydroxymethyl) amino-methane] acetic acid with a pH value of 5.0.

Formation of capsomeres can be monitored by electron microscopy, and, in instances wherein capsomeres are comprised of fusion
25 proteins, the presence of various protein components in the assembled capsomere can be confirmed by Western blot analysis using specific antisera.

According to the present invention, methods are provided for therapeutic treatment of individuals infected with HPV comprising the step
30 of administering to a patient in need thereof an amount of a vaccine

formulation of the invention effective to reduce the level of HPV infection. The invention also provide methods for prophylactic treatment of individuals susceptible to HPV infection comprising the step of administering to an individual susceptible to HPV infection an amount of a vaccine formulation of the invention effective to prevent HPV infection. While infected individuals can be easily identified using standard diagnostic techniques, susceptible individuals may be identified, for example, as those engaged in sexual relations with an infected individual. However, due to the high frequency of HPV infection, all sexually active persons are susceptible to papilloma virus infection.

Administration of a vaccine formulation can include one or more additional components such as pharmaceutically acceptable carriers, diluents, adjuvants, and/or buffers. Vaccines may be administered at a single time or at multiple times. Vaccine formulation of the invention may be delivered by various routes including, for example, oral, intravenous, intramuscular, nasal, rectal, transdermal, vaginal, subcutaneous, and intraperitoneal administration.

Vaccine formulations of the invention offer numerous advantages when compared to conventional vaccine preparations. As part of a therapeutic vaccination, capsomeres can promote elimination of persistently infected cells in, for example, patients with CIN or cervical carcinoma. Additionally, therapeutic vaccinations of this type can also serve a prophylactic purpose in protecting patients with CIN lesions from re-infection. As an additional advantage, capsomeres can escape neutralization by pre-existing anticapsid antibodies and thereby possess longer circulating half-life as compared to chimeric virus-like particles.

Vaccine formulations comprising chimeric capsomeres can provide the additional advantage of increased antigenicity of both protein components of the fusion protein from which the capsomere is formed. For example, in a VLP, protein components of the underlying capsomere

may be buried in the overall structure as a result of internalized positioning within the VLP itself. Similarly; epitopes of the protein components may be sterically obstructed as a result of capsomere-to-capsomere contact, and therefore inaccessible for eliciting an immune response. Preliminary
5 results using L1/E7 fusion proteins to produce VLPs support this position in that no antibody response was detected against the E7 component. This observation is consistent with previous results which indicate that the carboxy terminal region of L1 forms inter-pentameric arm structures that allow assembly of capsomeres into capsids [Garcia, *et al.*, *J. Virol.* 71:
10 2988-2995 (1997)]. Presumably in a chimeric capsomere structure, both protein components of the fusion protein substructure are accessible to evoke an immune response. Capsomere vaccines would therefore offer the additional advantage of increased antigenicity against any protein component, including, for example, neutralizing epitopes from other virus
15 proteins, expressed as a fusion with L1 amino acid sequences.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is illustrated by the following examples. Example 1 describes construction of expression vectors to produce fusion, or chimeric, viral proteins. Example 2 relates to
20 generation of recombinant baculoviruses for expression of viral proteins. Example 3 addresses purification of capsomeres. Example 4 describes an immunization protocol for production of antisera and monoclonal antibodies. Example 5 provides a peptide ELISA to quantitate capsomere formation. Example 6 describes an antigen capture ELISA to quantitate
25 capsomere formation. Example 7 provides a hemagglutinin assay to assay for the induction of neutralizing antibodies.

The resulting PCR product was digested with *EcoRV* to provide complementary ends and the digestion product circularized by ligation.

Ligated DNA was transformed into *E. coli* using standard techniques and plasmids from resulting colonies were screened for the presence of an *EcoRV* restriction site. One clone designated HPV 16 L1 Δ 310 was identified as having the appropriate twenty-seven nucleotide deletion and this construct was used to insert DNA fragments encoding other HPV 16 proteins at the *EcoRV* site as discussed below.

B. Generation of an HPV 16 L1 Δ C expression constructs

Two primers (SEQ ID NOs: 7 and 8) were designed complementary to the HPV 16 L1 open reading frame such that the primers abutted each other to permit amplification in reverse directions on the template DNA comprising HPV 16 L1-encoding sequences in pUC19 described above.

AAAGATATCTTGTAGTAAAAATTTGCGTCCTAAAGGAAAC
SEQ ID NO: 7

AAAGATATCTAATCTACCTCTACAACTGCTAAACGCAAAAAACG
SEQ ID NO: 8

Each primer introduced an *EcoRV* restriction site at the terminus of the amplification product. In the downstream primer (SEQ ID NO: 8), the *EcoRV* site was followed by a TAA translational stop codon positioned such that the amplification product, upon ligation of the *EcoRV* ends to circularize, would include deletion of the 34 carboxy terminal L1 amino acids. PCR was performed to amplify the partial L1 open reading frame and the complete vector. The amplification product was cleaved with *EcoRV*, circularized with T4 DNA ligase, and transformed into *E. coli* DH5 α cells. Plasmids from viable clones were analyzed for the presence of an *EcoRV* site which would linearize the plasmid. One positive

construct designated pUCHPV16L1ΔC was identified and used to insert DNA from other HPV 16 proteins utilizing the *EcoRV* site.

C. Insertion of DNA fragments into HPV 16 L1 Δ310 and HPV16L1ΔC

DNA fragments of HPV 16 E7 encoding amino acids 1-50,
5 1-60. 1-98, 25-75, 40-98, 50-98 in SEQ ID NO: 4 were amplified using
primers that introduced terminal 5' *EcoRV* restriction sites in order to
facilitate insertion of the fragment into either HPV 16 L1 Δ310 and
HPV16L1ΔC modified sequence. In the various amplification reactions,
primer E7.1 (SEQ ID NO: 9) was used in combination with primer E7.2
10 (SEQ ID NO: 10) to generate a DNA fragment encoding E7 amino acids 1-
50: with primer E7.3 (SEQ ID NO: 11) generate a DNA fragment
encoding E7 amino acids 1-60: or with primer E7.4 (SEQ ID NO: 12)
generate a DNA fragment encoding E7 amino acids 1-98. In other
amplification reactions, primer pairs E7.5 (SEQ ID NO: 13) and E7.6
15 (SEQ ID NO: 14) were used to amplify a DNA fragment encoding E7
amino acids 25-75: E7.7 (SEQ ID NO: 15) and E7.4 (SEQ ID NO: 12)
were used to amplify a DNA fragment encoding E7 amino acids 40-98; and
E7.8 (SEQ ID NO: 16) and E7.4 (SEQ ID NO: 12) were used to amplify a
DNA fragment encoding E7 amino acids 50-98.

20 Primer E7.1 SEQ ID NO: 9
AAAAGATATCATGCATGGAGATACACCTACATTGC

Primer E7.2 SEQ ID NO: 10
TTTGTATATCGGCTCTGTCCGGTTCTGCTTGTC

25 Primer E7.3 SEQ ID NO: 11
TTTGTATATCCTTGCAACAAAAGGTTACAATATTGTAATGGGCC

Primer E7.4 SEQ ID NO: 12
AAAAGATATCTGGTTTCTGAGAACAGATGGGGCAC

Primer E7.5 SEQ ID NO: 13
TTTTGATATCGATTATGAGCAATTAAATGACAGCTCAG

5 Primer E7.6 SEQ ID NO: 14
TTTTGATATCGTCTACGTGTGTGCTTTGTACGCAC

Primer E7.7 SEQ ID NO: 15
TTTATCGATATCGGTCCAGCTGGACAAGCAGAACCGGAC

10 Primer E7.8 SEQ ID NO: 16
TTTTGATATCGATGCCATTACAATATTGTAACCTTTG

Similarly, nucleotides from DNA encoding the influenza matrix protein (SEQ ID NO: 17) was amplified using the primer pair set out in SEQ ID NOs: 19 and 20. Both primers introduced an *EcoRV* restriction site in the amplification product.

15 TTTTGATATCGATATGGAATGGCTAAAGACAAGACCAATC
SEQ ID NO: 19

TTTTGATATCGTTGTTTGGATCCCCATTCCCATG
SEQ ID NO: 20

20 PCR products from each amplification reaction were cleaved with *EcoRV* and inserted into the *EcoRV* site of either the HPV 16 L1 Δ 310 and HPV16L1 Δ C sequences previously linearized with the same enzyme. In order to determine the orientation of inserts in plasmids encoding E7 amino acids 25-75 and 50-98 and plasmid including influenza matrix protein, *ClaI* digestion was employed, taking advantage of a

restriction site overlapping the newly created *EcoRV* restriction site
(GATATCGAT) and included in the upstream primer. For the three
expression constructs including the initiating methionine of HPV16 E7,
insert orientation was determined utilizing a *NsiI* restriction site within the
5 E7 coding region.

Once expression constructs having appropriate inserts were
identified, the protein coding region for both L1 and inserted amino acids
was excised as a unit using restriction enzymes *XbaI* and *SmaI* and the
isolated DNA ligated into plasmid pVL1393 (Invitrogen) to generate
10 recombinant baculoviruses.

D. Elimination of *EcoRV* Restriction Sites in Expression Constructs

The HPV 16 L1 Δ C sequence includes DNA from the
EcoRV site that results in translation of amino acids not normally found in
wild-type L1 polypeptides. Thus, a series of expression constructions was
15 designed in which the artificial *EcoRV* site was eliminated. The L1
sequence for this series of expression constructs was designated HPV
16L1 Δ C*.

To generate an expression construct containing the HPV
16L1 Δ C* sequence, two PCR reactions were performed to amplify two
20 overlapping fragments from the pUC-HPV16 L1 Δ C encoding E7 amino
acids 1-50. The resulting DNA fragments overlapped at the position of the
L1/E7 boundary but did not contain the two *EcoRV* restriction sites.
Fragment 1 was generated using primers P1 (SEQ ID NO: 21) and P2
(SEQ ID NO: 22) and fragment 2 using primers P3 (SEQ ID NO: 23) and
25 P4 (SEQ ID NO: 24).

Primer P1

GTTATGACATACATATTCTATG

SEQ ID NO: 21

Primer P2

SEQ ID NO: 22

CCATGCATTCCTGCTTGTAGTAAAAATTTGCGTCC

Primer P3

SEQ ID NO: 23

CTACAAGCAGGAATGCATGGAGATACACC

Primer P4

SEQ ID NO: 24

5 CATCTGAAGCTTAGTAATGGGCTCTGTCCGGTTCTG

Following the first two amplification reactions, the two purified products were used as templates in another PCR reaction using primers P1 and P4 only. The resulting amplification product was digested with enzymes *Eco*NI and *Hind*III inserted into the HPV 16L1ΔC expression construct described above following digestion with the same enzymes. The resulting expression construct differed from the original HPV16L1ΔC construct with DNA encoding L1 and E7 amino acids 1-50 by loss of the two internal *Eco*RV restriction sites. The first *Eco*RV site was replaced by DNA encoding native L1 alanine and glycine amino acids in this position and the second was replaced by a translational stop signal. In addition, the expression construct, designated HPV 16 L1ΔC* E7 1-52, contained the first 52 amino acids of HPV 16 E7 as a result of using primer P4 which also encodes E7 amino acids residues histidine at position 51 and tyrosine at position 52. HPV 16 L1ΔC* E7 1-52 was then used to generate additional HPV 16 L1ΔC expression constructs further including DNA encoding E7 amino acids 1-55 using primer P1 (SEQ ID NO: 21) in combination with primer P5 (SEQ ID NO: 25), E7 amino acids 1-60 with primer pair P1 and P6 (SEQ ID NO: 26), and E7 amino acids 1-65 with primer pair P1 and P7 (SEQ ID NO: 27). The additional amino acid-encoding DNA sequences in the amplification products arose from design of the primers to include additional nucleotides for the desired amino acids.

Primer P5 SEQ ID NO: 25
CATCTGAAGCTTAACAATATTGTAATGGGCTCTGTCCG

5 Primer P6 SEQ ID NO: 26
CATCTGAAGCTTACTTGCAACAAAAGGTTA-
CAATATTGTAATGGGCTCTGTCCG

Primer P7 SEQ ID NO: 27
CATCTGAAGCTTAAAGCGTAGAGTCACACTTGCAAC-
AAAAGGTTACAATATTGTAATGGGCTCTGTCCG

10 Similarly, HPV 16 L1 Δ C* E7 1-70 was generated using template DNA
encoding HPV 16 L1 Δ C* E7 1-66 and the primer pair P1 and P8 (SEQ ID
NO: 28).

15 Primer P8 SEQ ID NO: 28
CATCTGAAGCTTATTGTACGCACAAC-
CGAAGCGTAGAGTCACACTTG

20 Following each PCR reaction, the amplification products were digested
with *Eco*NI and *Hind*III and inserted into HPV16L1 Δ C previously digested
with the same enzymes. Sequences of each constructs were determined
using an Applied Biosystems Prism 377 sequencing instrument with
fluorescent chain terminating dideoxynucleotides [Prober *et al.*, *Science*
238:336-341 (1987)].

Example 2 Generation of Recombinant Baculoviruses

25 *Spodoptera frugiperda* (Sf9) cells were grown in suspension or
monolayer cultures at 27° in TNMFH medium (Sigma) supplemented with
10% fetal calf serum and 2 mM glutamine. For HPV 16 L1-based
recombinant baculovirus construction, Sf9 cells were transfected with 10 μ g
of transfer plasmid together with 2 μ g of linearized Baculo-Gold DNA

(PharMingen, San Diego, CA). Recombinant viruses were purified by according to manufacturer's suggested protocol.

To test for expression of HPV 16 L1 protein, 10^5 Sf9 cells were infected with baculovirus recombinant at a multiplicity of infection (m.o.i) of 5 to 10. After incubation for three to four days at 28°C, media was removed and cells were washed with PBS. The cells were lysed in SDS sample buffer and analyzed by SDS-PAGE and Western blotting using anti-HPV16 L1 and anti-HPV16 E7 antibodies.

In order to determine which of the chimeric L1 protein expression constructs would preferentially produce capsomeres, extracts from transfected cells were subjected to gradient centrifugation. Fractions obtained from the gradient were analyzed for L1 protein content by Western blotting and for VLP formation by electron microscopy. The results are shown in Table 1.

The intact HPV L1 protein, as well as the expression products HPV 16 L1 Δ 310 and HPV 16 L1 Δ C, each were shown to produce capsomeres and virus-like particles in equal proportions. When E7 coding sequences were inserted into the HPV 16 L1 Δ 310 vector, only fusion proteins including E7 amino acids 1 to 50 produced gave rise to detectable capsomere formation.

When E7 encoding DNA was inserted into the HPV 16 L1 Δ C vector, all fusion proteins were found to produce capsomeres; chimeric proteins including E7 amino acid residues 40-98 produced the highest level of exclusively capsomere structures. Chimeric proteins including E7 amino acids 1-98 and 25-75 both produced predominantly capsomeres, even thorough virus-like particle formation was also observed. The chimeric protein including E7 amino acids 1-60 resulted in nearly equal levels of capsomere and virus-like particle production.

When E7 sequences were inserted into the HPV 16 L1 Δ *C vector, all fusion proteins were shown to produce capsomeres. Insertion of

DNA encoding E7 residues 1-52, 1-55, and 1-60 produced the highest level of capsomeres, but equal levels of virus-like particle production were observed. While insertion of DNA encoding E7 DNA for residues 1-65, 1-70, 25-75, 40-98, and 1-98 resulted in comparatively lower levels or undetectable levels of capsid, capsomeres were produced in high quantities.

TABLE 1
Capsomere and Capsid Forming Capacity of
Chimeric HPV L1 Proteins

	<u>L1 Expression Construct</u>	<u>Insert</u>	<u>Capsomere Yield</u>	<u>Capsid Yield</u>
10	HVP 16 L1	None	+++++	+++++
	HPV 16 L1Δ310	None	+++	++
	HPV 16 L1ΔC	None	++++	++++
	HPV 16 L1Δ310	E7 1-98	-	-
15	HPV 16 L1Δ310	E7 1-50	++	-
	HPV 16 L1Δ310	E7 25-75	-	-
	HPV 16 L1Δ310	E7 50-98	-	-
	HPV 16 L1ΔC	E7 1-98	+++	+
	HPV 16 L1ΔC	E7 25-75	+++	+
20	HPV 16 L1ΔC	E7 50-98	+	+
	HPV 16 L1ΔC	E7 1-60	+++++	+++++
	HPV 16 L1ΔC	E7 40-98	++++	-
	HPV 16 L1ΔC	Influenza	+++	+
	HPV 16 L1Δ*C	E7 1-52	+++++	+++++
25	HPV 16 L1Δ*C	E7 1-55	+++++	+++++
	HPV 16 L1Δ*C	E7 1-60	+++	++++
	HPV 16 L1Δ*C	E7 1-65	++	-
	HPV 16 L1Δ*C	E7 1-70	++	-

Example 3 Purification of Capsomeres

Trichopulsia ni (TN) High Five cells were grown to a density of approximately 2×10^6 cells/ml in Ex-Cell 405 serum-free medium (JRH Biosciences). Approximately 2×10^8 cells were pelleted by centrifugation at 1000 x g for 15 minutes, resuspended in 20 ml of medium, and infected with recombinant baculoviruses at m.o.i of 2 to 5 for 1 hour at room temperature. After addition of 200 ml medium, cells were plated and incubated for 3 to 4 days at 27°C. Following incubation, cells were harvested, pelleted, and resuspended in 10 ml of extraction buffer.

The following steps were performed at 4°C. Cells were sonicated for 45 seconds at 60 watts and the resulting cell lysate was centrifuged at 10,000 rpm in a Sorval SS34 rotor. The supernatant was removed and retained while the resulting pellet was resuspended in 6 ml of extraction buffer, sonicated for an additional 3 seconds at 60 watts, and centrifuged again. The two supernatants were combined, layered onto a two-step gradient containing 14 ml of 40% sucrose on top of 8 ml of CsCl solution (4.6 g CsCl per 8 ml in extraction buffer), and centrifuged in a Sorval AH629 swinging bucket rotor for 2 hours at 27,000 rpm at 10°C. The interface region between the CsCl and the sucrose along with the CsCl complete layer were collected into 13.4 ml Quickseal tubes (Beckman) and extraction buffer added to adjust the volume 13.4 ml. Samples were centrifuged overnight at 50,000 rpm at 20°C in a Beckman 70 TI rotor. Gradients were fractionated (1 ml per fraction) by puncturing tubes on top and bottom with a 21-gauge needle. Fractions were collected from each tube and 2.5 µl of each fraction were analyzed by a 10% SDS-polyacrylamide gel and Western blotting using an anti-HPV16 L1 antibody.

Virus-like particles and capsomeres were separated from the fractions identified above by sedimentation on 10 to 50% sucrose gradients. Peak fractions from CsCl gradients were pooled and dialyzed for 2 hours against 5 mM HEPES (pH 7.5). Half of the dialysate was used to produce capsomeres by disassembly of intact VLPs overnight by adding EDTA (final concentration 50 mM), EGTA (50

mM), DTT (30 mM). NaCl (100 mM), and Tris/HCl, pH 8.0, (10 mM). As control, NaCl and Tris/HCl only were added to the other half.

For analysis of capsomeres produced from disassembled VLPs, EDTA, EGTA, and DTT (final concentration 5 mM each) were added to the
5 sucrose cushions which were centrifuged at 250,000 x *g* for 2 to 4 hours at 4°C. Fractions were collected by puncturing tubes from the bottom. A 1:10 dilution of each fraction was then analyzed by antigen capture ELISA.

Example 4 **Immunization Protocol for Production of** **Polyclonal Antisera and Monoclonal Antibodies**

10 Balb/c mice are immunized subcutaneously three times, every four weeks with approximately 60 µg of HPV chimeric capsomeres mixed 1:1 with complete or incomplete Freund's Adjuvants in a total volume of 100 µl. Six weeks after the third immunization, mice are sacrificed and blood is collected by
15 cardiac puncture.

Example 5 **Peptide ELISA to Quantitate Capsomere Formation**

Microtiter plates (Dynatech) are coated overnight with 50 µl of peptide E701 [Muller *et al.*, 1982] at a concentration of 10 µg/ml in PBS. Wells
20 are blocked for 2 hour at 37°C with 100 µl of buffer containing 5% BSA and 0.05% Tween 20 in PBS and washed three times with PBS containing 0.05% Tween 20. After the third wash, 50 µl of sera diluted 1:5000 in BSA/Tween 20/PBS is added to each well and incubation carried out for 1 hour. Plates are washed again as before and 50 µl of goat-anti-mouse peroxidase conjugate is added
25 at a 1:5000 dilution. After 1 hour, plates are washed and stained using ABTS substrate (0.2 mg/ml, 2,2'-Azino-bis(3-ethylbenzthiazoline-β-sulfonic acid in 0.1 M Na-Acetate-Phosphate buffer (pH 4.2) with 4 µl 30% H₂O₂ per 10 ml). Extinction is measured after 1 hour at 490 nm in a Dynatech automated plate reader.

Example 6
Antigen Capture ELISA to Quantitate Capsomere Formation

To allow relative quantification of virus-like particles and capsomeres in fractions of CsCl gradients, an antigen capture ELISA was utilized.

5 Microtiter plates were coated overnight with 50 μ l/well of a 1:500 dilution (final concentration of 2 μ g per ml, in PBS) with a protein A purified mouse monoclonal antibody immunospecific for HPV 16 L1 (antibodies 25/C, MM07 and Ritti 1 were obtained from mice immunized with HPV 16 VLPs). Plates were blocked with 5% milk/PBS for 1 hour and 50 μ l of fractions of CsCl gradients were added

10 for 1 hour at 37°C using a 1:300 dilution (in 5% milk/PBS). After three washings with PBS/0.05% Tween 20, 50 μ l of a polyclonal rabbit antiserum (1:3000 dilution in milk/PBS), raised against HPV 16 VLPs was added and plates were incubated at 37° for 1 hour. Plates were washed again and further incubated with 50 μ l of a goat-anti-rabbit peroxidase conjugate (Sigma) diluted 1:5000 in PBS

15 containing 5% milk for 1 hour. After final washing, plates were stained with ABTS substrate for 30 minutes and extinction measured at 490 nm in a Dynatech automated plate reader. As a negative control, the assay also included wells coated only with PBS.

To test monoclonal antibodies for capsomere specificity, VLPs with

20 EDTA/DTT to disassemble particles. Treated particle preparations were assayed in the antigen-capture ELISA and readings compared to untreated controls. For disassembly, 40 μ l of VLPs was incubated overnight at 4°C in 500 μ l of disruption buffer containing 30 mM DTT, 50 mM EGTA, 60 mM EDTA, 100 mM NaCl, and 100 mM Tris/HCl, pH 8.0. Aliquots of treated and untreated

25 particles were used in the above capture ELISA in a 1:20-1:40 dilution.

Example 7
Hemagglutinin Inhibition Assay

In order to determine the extent to which chimeric capsomere vaccines evoke production of neutralizing antibodies, a hemagglutination inhibition

30 assay is carried out as briefly described below. This assay is based on previous

observations that virus-like particles are capable of hemagglutinating red blood cells.

Mice are immunized with any of a chimeric capsomere vaccine and sera is collected as described above in Example 4. As positive controls, HPV16 L1 virus like particles (VLPs) and bovine PV1 (BPV) L1 VLPs are assayed in parallel with a chimeric capsomere preparation. To establish a positive baseline, the HPV16 or BPV1 VLPs are first incubated with or without sera collected from immunized mice after which red blood cells are added. The extent to which preincubation with mouse sera inhibits red blood cell hemagglutination is an indication of the neutralizing capacity of the mouse sera. The experiments are then repeated using chimeric capsomeres in order to determine the neutralizing effect of the mouse sera on the vaccine. A brief protocol for the hemagglutination inhibition assay is described below.

One hundred microliters of heparin (1000 usp units/ml) are added to 1 ml fresh mouse blood. Red blood cells are washed three times with PBS followed by centrifugation and resuspension in a volume of 10 ml. Next, erythrocytes are resuspended in 0.5 ml PBS and stored at 4°C for up to three days. For the hemagglutinin assay, 70 μ l of the suspension is used per well on a 96-well plate.

Chimeric capsomere aliquots from CsCl gradients are dialyzed for one hour against 10 mM Hepes (pH 7.5) and 100 μ l of two-fold serial dilutions in PBS are added to mouse erythrocytes in round-bottom 96-well microtiter plates which are further incubated for 3-16 hours at 4°C. For hemagglutination inhibition, capsomeres are incubated with dilutions of antibodies in PBS for 60 minutes at room temperature and then added to the erythrocytes. The level of erythrocyte hemagglutination, and therefore the presence of neutralizing antibodies, is determined by standard methods.

In preliminary results, mouse sera generated against chimeric capsomeres comprising HPV16L1 Δ C protein in association with E7 amino acid residues 1-98 was observed to inhibit hemagglutination by HPV16 VLPs, but not

by BPV VLPs. The mouse sera was therefore positive for neutralizing antibodies against the human VLPs and this differential neutralization was most likely the result of antibody specificity for epitopes against which the antibodies were raised.

5 Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
- (ii) TITLE OF INVENTION: Papilloma Virus Capsomere Vaccine Formulations and Methods of Use
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
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 - (C) CITY: Chicago
 - (D) STATE: Illinois
 - (E) COUNTRY: United States of America
 - (F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Williams Jr., Joseph A.
 - (B) REGISTRATION NUMBER: 38,659
 - (C) REFERENCE/DOCKET NUMBER: 27013/34028
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 312-474-6300
 - (B) TELEFAX: 312-474-0448

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1518 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..1518

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG	TCT	CTT	TGG	CTG	CCT	AGT	GAG	GCC	ACT	GTC	TAC	TTG	CCT	CCT	GTC	
Met	Ser	Leu	Trp	Leu	Pro	Ser	Glu	Ala	Thr	Val	Tyr	Leu	Pro	Pro	Val	
1					5				10					15		
CCA	GTA	TCT	AAG	GTT	GTA	AGC	ACG	GAT	GAA	TAT	GTT	GCA	CGC	ACA	AAC	
Pro	Val	Ser	Lys	Val	Val	Ser	Thr	Asp	Glu	Tyr	Val	Ala	Arg	Thr	Asn	
			20					25					30			

48

96

ATA	TAT	TAT	CAT	GCA	GGA	ACA	TCC	AGA	CTA	CTT	GCA	GTT	GGA	CAT	CCC	144
Ile	Tyr	Tyr	His	Ala	Gly	Thr	Ser	Arg	Leu	Leu	Ala	Val	Gly	His	Pro	
		35					40					45				
TAT	TTT	CCT	ATT	AAA	AAA	CCT	AAC	AAT	AAC	AAA	ATA	TTA	GTT	CCT	AAA	192
Tyr	Phe	Pro	Ile	Lys	Lys	Pro	Asn	Asn	Asn	Lys	Ile	Leu	Val	Pro	Lys	
	50					55					60					
GTA	TCA	GGA	TTA	CAA	TAC	AGG	GTA	TTT	AGA	ATA	CAT	TTA	CCT	GAC	CCC	240
Val	Ser	Gly	Leu	Gln	Tyr	Arg	Val	Phe	Arg	Ile	His	Leu	Pro	Asp	Pro	
	65				70					75					80	
AAT	AAG	TTT	GGT	TTT	CCT	GAC	ACC	TCA	TTT	TAT	AAT	CCA	GAT	ACA	CAG	288
Asn	Lys	Phe	Gly	Phe	Pro	Asp	Thr	Ser	Phe	Tyr	Asn	Pro	Asp	Thr	Gln	
				85					90					95		
CGG	CTG	GTT	TGG	GCC	TGT	GTA	GGT	GTT	GAG	GTA	GGT	CGT	GGT	CAG	CCA	336
Arg	Leu	Val	Trp	Ala	Cys	Val	Gly	Val	Glu	Val	Gly	Arg	Gly	Gln	Pro	
			100					105					110			
TTA	GGT	GTG	GGC	ATT	AGT	GGC	CAT	CCT	TTA	TTA	AAT	AAA	TTG	GAT	GAC	384
Leu	Gly	Val	Gly	Ile	Ser	Gly	His	Pro	Leu	Leu	Asn	Lys	Leu	Asp	Asp	
		115					120					125				
ACA	GAA	AAT	GCT	AGT	GCT	TAT	GCA	GCA	AAT	GCA	GGT	GTG	GAT	AAT	AGA	432
Thr	Glu	Asn	Ala	Ser	Ala	Tyr	Ala	Ala	Asn	Ala	Gly	Val	Asp	Asn	Arg	
	130					135					140					
GAA	TGT	ATA	TCT	ATG	GAT	TAC	AAA	CAA	ACA	CAA	TTG	TGT	TTA	ATT	GGT	480
Glu	Cys	Ile	Ser	Met	Asp	Tyr	Lys	Gln	Thr	Gln	Leu	Cys	Leu	Ile	Gly	
	145				150					155					160	
TGC	AAA	CCA	CCT	ATA	GGG	GAA	CAC	TGG	GGC	AAA	GGA	TCC	CCA	TGT	ACC	528
Cys	Lys	Pro	Pro	Ile	Gly	Glu	His	Trp	Gly	Lys	Gly	Ser	Pro	Cys	Thr	
				165					170					175		
AAT	GTT	GCA	GTA	AAT	CCA	GGT	GAT	TGT	CCA	CCA	TTA	GAG	TTA	ATA	AAC	576
Asn	Val	Ala	Val	Asn	Pro	Gly	Asp	Cys	Pro	Pro	Leu	Glu	Leu	Ile	Asn	
			180					185					190			
ACA	GTT	ATT	CAG	GAT	GGT	GAT	ATG	GTT	GAT	ACT	GGC	TTT	GGT	GCT	ATG	624
Thr	Val	Ile	Gln	Asp	Gly	Asp	Met	Val	Asp	Thr	Gly	Phe	Gly	Ala	Met	
		195					200					205				
GAC	TTT	ACT	ACA	TTA	CAG	GCT	AAC	AAA	AGT	GAA	GTT	CCA	CTG	GAT	ATT	672
Asp	Phe	Thr	Thr	Leu	Gln	Ala	Asn	Lys	Ser	Glu	Val	Pro	Leu	Asp	Ile	
	210					215					220					
TGT	ACA	TCT	ATT	TGC	AAA	TAT	CCA	GAT	TAT	ATT	AAA	ATG	GTG	TCA	GAA	720
Cys	Thr	Ser	Ile	Cys	Lys	Tyr	Pro	Asp	Tyr	Ile	Lys	Met	Val	Ser	Glu	
	225				230					235					240	
CCA	TAT	GGC	GAC	AGC	TTA	TTT	TTT	TAT	TTA	CGA	AGG	GAA	CAA	ATG	TTT	768
Pro	Tyr	Gly	Asp	Ser	Leu	Phe	Phe	Tyr	Leu	Arg	Arg	Glu	Gln	Met	Phe	
				245					250					255		
GTT	AGA	CAT	TTA	TTT	AAT	AGG	GCT	GGT	GCT	GTT	GGT	GAA	AAT	GTA	CCA	816
Val	Arg	His	Leu	Phe	Asn	Arg	Ala	Gly	Ala	Val	Gly	Glu	Asn	Val	Pro	
			260					265					270			
GAC	GAT	TTA	TAC	ATT	AAA	GGC	TCT	GGG	TCT	ACT	GCA	AAT	TTA	GCC	AGT	864
Asp	Asp	Leu	Tyr	Ile	Lys	Gly	Ser	Gly	Ser	Thr	Ala	Asn	Leu	Ala	Ser	
		275					280					285				

TCA Ser 290	AAT Asn	TAT Tyr	TTT Phe	CCT Pro	ACA Thr	CCT Pro 295	AGT Ser	GGT Gly	TCT Ser	ATG Met	GTT Val 300	ACC Thr	TCT Ser	GAT Asp	GCC Ala	912
CAA Gln 305	ATA Ile	TTC Phe	AAT Asn	AAA Lys	CCT Pro 310	TAT Tyr	TGG Trp	TTA Leu	CAA Gln 315	CGA Arg	GCA Ala	CAG Gln	GGC Gly	CAC His	AAT Asn 320	960
AAT Asn	GGC Gly	ATT Ile	TGT Cys	TGG Trp 325	GGT Gly	AAC Asn	CAA Gln	CTA Leu	TTT Phe 330	GTT Val	ACT Thr	GTT Val	GTT Val	GAT Asp 335	ACT Thr	1008
ACA Thr	CGC Arg	AGT Ser	ACA Thr 340	AAT Asn	ATG Met	TCA Ser	TTA Leu	TGT Cys 345	GCT Ala	GCC Ala	ATA Ile	TCT Ser	ACT Thr 350	TCA Ser	GAA Glu	1056
ACT Thr	ACA Thr	TAT Tyr 355	AAA Lys	AAT Asn	ACT Thr	AAC Asn	TTT Phe 360	AAG Lys	GAG Glu	TAC Tyr	CTA Leu 365	CGA Arg	CAT His	GGG Gly	GAG Glu	1104
GAA Glu 370	TAT Tyr	GAT Asp	TTA Leu	CAG Gln	TTT Phe 375	ATT Ile	TTT Phe	CAA Gln	CTG Leu	TGC Cys	AAA Lys 380	ATA Ile	ACC Thr	TTA Leu	ACT Thr	1152
GCA Ala 385	GAC Asp	GTT Val	ATG Met	ACA Thr 390	TAC Tyr	ATA Ile	CAT His	TCT Ser	ATG Met	AAT Asn 395	TCC Ser	ACT Thr	ATT Ile	TTG Leu 400	GAG Glu	1200
GAC Asp	TGG Trp	AAT Asn	TTT Phe 405	GGT Gly	CTA Leu	CAA Gln	CCT Pro	CCC Pro	CCA Pro 410	GGA Gly	GGC Gly	ACA Thr	CTA Leu 415	GAA Glu	GAT Asp	1248
ACT Thr	TAT Tyr	AGG Arg	TTT Phe 420	GTA Val	ACC Thr	TCC Ser	CAG Gln	GCA Ala 425	ATT Ile	GCT Ala	TGT Cys	CAA Gln	AAA Lys 430	CAT His	ACA Thr	1296
CCT Pro	CCA Pro	GCA Ala 435	CCT Pro	AAA Lys	GAA Glu	GAT Asp	CCC Pro 440	CTT Leu	AAA Lys	AAA Lys	TAC Tyr 445	ACT Thr	TTT Phe	TGG Trp	GAA Glu	1344
GTA Val 450	AAT Asn	TTA Leu	AAG Lys	GAA Glu	AAG Lys	TTT Phe 455	TCT Ser	GCA Ala	GAC Asp	CTA Leu 460	GAT Asp	CAG Gln	TTT Phe	CCT Pro	TTA Leu	1392
GGA Gly 465	CGC Arg	AAA Lys	TTT Phe	TTA Leu	CTA Leu 470	CAA Gln	GCA Ala	GGA Gly	TTG Leu	AAG Lys 475	GCC Ala	AAA Lys	CCA Pro	AAA Lys	TTT Phe 480	1440
ACA Thr	TTA Leu	GGA Gly	AAA Lys	CGA Arg 485	AAA Lys	GCT Ala	ACA Thr	CCC Pro	ACC Thr 490	ACC Thr	TCA Ser	TCT Ser	ACC Thr	TCT Ser	ACA Thr	1488
ACT Thr	GCT Ala	AAA Lys	CGC Arg	AAA Lys	AAA Lys	CGT Arg	AAG Lys	CTG Leu	TAA *							1518
				500				505								

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 506 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear



Met 1	Ser	Leu	Trp	Leu 5	Pro	Ser	Glu	Ala	Thr 10	Val	Tyr	Leu	Pro	Pro 15	Val
Pro	Val	Ser	Lys 20	Val	Val	Ser	Thr	Asp 25	Glu	Tyr	Val	Ala	Arg 30	Thr	Asn
Ile	Tyr	Tyr 35	His	Ala	Gly	Thr	Ser 40	Arg	Leu	Leu	Ala	Val 45	Gly	His	Pro
Tyr	Phe 50	Pro	Ile	Lys	Lys	Pro 55	Asn	Asn	Asn	Lys	Ile 60	Leu	Val	Pro	Lys
Val 65	Ser	Gly	Leu	Gln	Tyr 70	Arg	Val	Phe	Arg	Ile 75	His	Leu	Pro	Asp	Pro 80
Asn	Lys	Phe	Gly	Phe 85	Pro	Asp	Thr	Ser	Phe 90	Tyr	Asn	Pro	Asp	Thr 95	Gln
Arg	Leu	Val	Trp 100	Ala	Cys	Val	Gly	Val 105	Glu	Val	Gly	Arg	Gly 110	Gln	Pro
Leu	Gly 115	Val	Gly	Ile	Ser	Gly	His 120	Pro	Leu	Leu	Asn 125	Lys	Leu	Asp	Asp
Thr	Glu 130	Asn	Ala	Ser	Ala	Tyr 135	Ala	Ala	Asn	Ala	Gly 140	Val	Asp	Asn	Arg
Glu 145	Cys	Ile	Ser	Met	Asp 150	Tyr	Lys	Gln	Thr	Gln 155	Leu	Cys	Leu	Ile	Gly 160
Cys	Lys	Pro	Pro	Ile 165	Gly	Glu	His	Trp	Gly 170	Lys	Gly	Ser	Pro	Cys 175	Thr
Asn	Val	Ala	Val 180	Asn	Pro	Gly	Asp	Cys 185	Pro	Pro	Leu	Glu	Leu 190	Ile	Asn
Thr	Val	Ile 195	Gln	Asp	Gly	Asp	Met 200	Val	Asp	Thr	Gly	Phe 205	Gly	Ala	Met
Asp	Phe 210	Thr	Thr	Leu	Gln	Ala 215	Asn	Lys	Ser	Glu	Val 220	Pro	Leu	Asp	Ile
Cys 225	Thr	Ser	Ile	Cys	Lys 230	Tyr	Pro	Asp	Tyr	Ile 235	Lys	Met	Val	Ser	Glu 240
Pro	Tyr	Gly	Asp	Ser 245	Leu	Phe	Phe	Tyr	Leu 250	Arg	Arg	Glu	Gln	Met 255	Phe
Val	Arg	His	Leu 260	Phe	Asn	Arg	Ala	Gly 265	Ala	Val	Gly	Glu	Asn 270	Val	Pro
Asp	Asp	Leu 275	Tyr	Ile	Lys	Gly	Ser 280	Gly	Ser	Thr	Ala	Asn 285	Leu	Ala	Ser
Ser	Asn 290	Tyr	Phe	Pro	Thr	Pro 295	Ser	Gly	Ser	Met	Val 300	Thr	Ser	Asp	Ala

Gln Ile Phe Asn Lys Pro Tyr Trp Leu Gln Arg Ala Gln Gly His Asn
305 310 315 320

Asn Gly Ile Cys Trp Gly Asn Gln Leu Phe Val Thr Val Val Asp Thr
325 330 335

Thr Arg Ser Thr Asn Met Ser Leu Cys Ala Ala Ile Ser Thr Ser Glu
340 345 350

Thr Thr Tyr Lys Asn Thr Asn Phe Lys Glu Tyr Leu Arg His Gly Glu
355 360 365

Glu Tyr Asp Leu Gln Phe Ile Phe Gln Leu Cys Lys Ile Thr Leu Thr
370 375 380

Ala Asp Val Met Thr Tyr Ile His Ser Met Asn Ser Thr Ile Leu Glu
385 390 395 400

Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu Asp
405 410 415

Thr Tyr Arg Phe Val Thr Ser Gln Ala Ile Ala Cys Gln Lys His Thr
420 425 430

Pro Pro Ala Pro Lys Glu Asp Pro Leu Lys Lys Tyr Thr Phe Trp Glu
435 440 445

Val Asn Leu Lys Glu Lys Phe Ser Ala Asp Leu Asp Gln Phe Pro Leu
450 455 460

Gly Arg Lys Phe Leu Leu Gln Ala Gly Leu Lys Ala Lys Pro Lys Phe
465 470 475 480

Thr Leu Gly Lys Arg Lys Ala Thr Pro Thr Thr Ser Ser Thr Ser Thr
485 490 495

Thr Ala Lys Arg Lys Lys Arg Lys Leu *
500 505

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 297 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..297

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA 48
 Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu Asp Leu Gln
 1 5 10 15

CCA GAG ACA ACT GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC TCA 96
 Pro Glu Thr Thr Asp Leu Tyr Cys Tyr Glu Gln Leu Asn Asp Ser Ser
 20 25 30

GAG GAG GAG GAT GAA ATA GAT GGT CCA GCT GGA CAA GCA GAA CCG GAC	144
Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala Glu Pro Asp	
35 40 45	
AGA GCC CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG TGT GAC TCT ACG	192
Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr	
50 55 60	
CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA	240
Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu	
65 70 75 80	
GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAG	288
Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln	
85 90 95	
AAA CCA TAA	297
Lys Pro *	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu Asp Leu Gln	
1 5 10 15	
Pro Glu Thr Thr Asp Leu Tyr Cys Tyr Glu Gln Leu Asn Asp Ser Ser	
20 25 30	
Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala Glu Pro Asp	
35 40 45	
Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr	
50 55 60	
Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu	
65 70 75 80	
Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln	
85 90 95	
Lys Pro *	

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCCGATATC GCCTTTAATG TATAAATCGT CTGG

34

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCCCGATATC TCAAATTATT TTCCTACACC TAGTG

35

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAAGATATCT TGTAGTAAAA ATTTGCGTCC TAAAGGAAAC

40

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 44 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAAGATATCT AATCTACCTC TACAACTGCT AAACGCAAAA AACG

44

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAAAGATATC ATGCATGGAG ATACACCTAC ATTGC

35

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTTGATATC GGCTCTGTCC GGTTCGTCTT GTCC

34

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTTTGATATC CTTGCAACAA AAGGTTACAA TATTGTAATG GGCC

44

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AAAAGATATC TGGTTTCTGA GAACAGATGG GGCAC

35

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTGATATC GATTATGAGC AATTAAATGA CAGCTCAG

38

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTTTGATATC GTCTACGTGT GTGCTTTGTA CGCAC

35

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 39 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTTATCGATA TCGGTCCAGC TGGACAAGCA GAACCGGAC

39

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 39 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTTTGATATC GATGCCCATT ACAATATTGT AACCTTTTG

39

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 294 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..294

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATG AGT CTT CTA ACC GAG GTC GAA ACG CTT ACC AGA AAC GGA TGG GAG 48
Met Ser Leu Leu Thr Glu Val Glu Thr Leu Thr Arg Asn Gly Trp Glu
1 5 10 15

TGC AAA TGC AGC GAT TCA AGT GAT CCT CTC ATT ATC GCA GCG AGT ATC 96
Cys Lys Cys Ser Asp Ser Ser Asp Pro Leu Ile Ile Ala Ala Ser Ile
20 25 30

ATT GGG ATC TTG CAC TTG ATA TTG TGG ATT TTT TAT CGT CTT TTC TTC 144
Ile Gly Ile Leu His Leu Ile Leu Trp Ile Phe Tyr Arg Leu Phe Phe
35 40 45

AAA TGC ATT TAT CGT CGC CTT AAA TAC GGT TTG AAA AGA GGG CCT TCT 192
Lys Cys Ile Tyr Arg Arg Leu Lys Tyr Gly Leu Lys Arg Gly Pro Ser
50 55 60

ACG GAA GGA GCG CCT GAG TCT ATG AGG GAA GAA TAT CGG CAG GAA CAG	240
Thr Glu Gly Ala Pro Glu Ser Met Arg Glu Glu Tyr Arg Gln Glu Gln	
65 70 75 80	
CAG AGT GCT GTG GAT GTT GAC GAT GTT CAT TTT GTC AAC ATA GAG CTG	288
Gln Ser Ala Val Asp Val Asp Asp Val His Phe Val Asn Ile Glu Leu	
85 90 95	
GAG TAA	294
Glu *	

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ser Leu Leu Thr Glu Val Glu Thr Leu Thr Arg Asn Gly Trp Glu	
1 5 10 15	
Cys Lys Cys Ser Asp Ser Ser Asp Pro Leu Ile Ile Ala Ala Ser Ile	
20 25 30	
Ile Gly Ile Leu His Leu Ile Leu Trp Ile Phe Tyr Arg Leu Phe Phe	
35 40 45	
Lys Cys Ile Tyr Arg Arg Leu Lys Tyr Gly Leu Lys Arg Gly Pro Ser	
50 55 60	
Thr Glu Gly Ala Pro Glu Ser Met Arg Glu Glu Tyr Arg Gln Glu Gln	
65 70 75 80	
Gln Ser Ala Val Asp Val Asp Asp Val His Phe Val Asn Ile Glu Leu	
85 90 95	
Glu *	

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTGATATC GATATGGAAT GGCTAAAGAC AAGACCAATC

40

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TTTTGATATC GTTGTGGG TCCCATTC CATTG

35

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTATGACAT ACATACATTC TATG

24

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCATGCATTC CTGCTGTAG TAAAATTG CGTCC

35

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTACAAGCAG GAATGCATGG AGATACACC

29

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CATCTGAAGC TTAGTAATGG GCTCTGTCCG GTTCTG

36

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CATCTGAAGC TTATCAATAT TGTAATGGGC TCTGTCCG

38

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 54 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CATCTGAAGC TTA CTTGCAA CAAAAGGTTA CAATATTGTA ATGGGCTCTG TCCG

54

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 69 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CATCTGAAGC TTAAAGCGTA GAGTCACACT TGCAACAAAA GGTTACAATA TTGTAATGGG

60

CTCTGTCCG

69

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 47 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CATCTGAAGC TTATTGTACG CACAACCGAA GCGTAGAGTC ACACTTG

47

WHAT IS CLAIMED IS:

1. A vaccine formulation comprising a human papilloma virus capsomere, said capsomere comprising a fusion protein comprising a human papilloma virus L1 protein adjacent amino acid residues from a second protein.

2. A vaccine formulation comprising a human papilloma virus capsomere, said capsomere comprising a truncated human papilloma virus L1 protein having a deletion of one or more amino acid residues necessary for formation of a virus-like particle.

3. The vaccine formulation of claim 2 wherein said capsomere comprises a fusion protein comprising a truncated human papilloma virus L1 protein adjacent amino acid residues from a second protein.

4. The vaccine formulation of any one of claims 1,2, or 3 wherein the L1 protein is encoded in the genome of a human papilloma virus selected from the group consisting of HPV6, HPV11, HPV16, HPV18, HPV33, HPV35, and HPV45.

5. The vaccine formulation of claim 4 wherein the papilloma virus is HPV16.

6. The vaccine formulation of any one of claims 2, 3, or 5 wherein carboxy terminal amino acid residues are deleted from the L1 protein.

7. The vaccine formulation of claim 6 wherein 1 to 34 carboxy terminal amino acid residues are deleted from the L1 protein.

8. The vaccine formulation of claim 7 wherein 34 carboxy terminal amino acid residues are deleted from the L1 protein.
9. The vaccine formulation of any one of claims 2, 3, or 5 wherein amino terminal amino acid residues are deleted from the L1 protein.
10. The vaccine formulation of any one of claims 2, 3, or 5 wherein internal amino acid residues are deleted from the L1 protein.
11. The vaccine formulation of claim 10 wherein the amino acid residues deleted from the L1 protein comprise a nuclear localization signal.
12. The vaccine formulation of claims 2 or 3 wherein the amino acids residues from the second protein are derived from an HPV protein.
13. The vaccine formulation of claim 12 wherein the HPV protein is an early HPV protein.
14. The vaccine formulation of claim 12 wherein the early HPV protein is selected from the group consisting of E1, E2, E3, E4, E5, E6, and E7.
15. A method of treating an individual infected with an HPV virus comprising the step of administering to a patient in need thereof an amount of the vaccine formulation of claims 1, 2, 3, 5, 7, 8, 11, 13 or 14 effective to reduce the level of HPV infection.

16. A method for preventing papilloma virus infection comprising the step of administering to an individual susceptible thereto an amount of the vaccine formulation of claims 1, 2, 3, 5, 7, 8, 11, 13 or 14 effective to inhibit HPV infection.

ABSTRACT

Vaccine formulations comprising viral capsomeres are disclosed along with methods for their production. Therapeutic and prophylactic methods of use for the vaccine formulations are also disclosed.

APPENDIX B

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PAPILLOMA VIRUS CAPSOMERE VACCINE FORMULATIONS AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to vaccine formulations
5 comprising papilloma virus proteins, either as fusion proteins, truncated
proteins, or truncated fusion proteins. The invention further embraces
methods for producing capsomeres of the formulations, as well as
prophylactic and therapeutic methods for their use.

BACKGROUND

10 Infections with certain high-risk strains of genital papilloma
viruses in humans (HPV) -- for example, HPV 16, 18, or 45 -- are
believed to be the main risk factor for the formation of malignant tumors of
the anogenital tract. Of the possible malignancies, cervical carcinoma is by
far the most frequent; according to an estimate by the World Health
15 Organization (WHO), almost 500,000 new cases of the disease occur
annually. Because of the frequency with which this pathology occurs, the
connection between HPV infection and cervical carcinoma has been
extensively examined, leading to numerous generalizations.

For example, precursor lesions of cervical intraepithelial
20 neoplasia (CIN) are known to be caused by papilloma virus infections
[Crum, *New Eng. J. Med.* 310:880-883 (1984)]. DNA from the genomes
of certain HPV types, including for example, strains 16, 18, 33, 35, and
45, have been detected in more than 95 % of tumor biopsies from patients
with this disorder, as well as in primary cell lines cultured from the
25 tumors. Approximately 50 to 70 % of the biopsied CIN tumor cells have
been found to include DNA derived only from HPV 16.

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The protein products of the HPV 16 and HPV 18 early genes
E6 and E7 have been detected in cervical carcinoma cell lines as well as in

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human keratinocytes transformed *in vitro* [Wettstein, *et al.*, in PAPILLOMA VIRUSES AND HUMAN CANCER, Pfister (Ed.), CRC Press: Boca Raton, FL 1990 pp 155-179] and a significant percentage of patients with cervical carcinoma have anti-E6 or anti-E7 antibodies. The E6 and E7 proteins
5 have been shown to participate in induction of cellular DNA synthesis in human cells, transformation of human keratinocytes and other cell types, and tumor formation in transgenic mice [Arbelt, *et al.*, *J. Virol.*, 68:4358-4364 (1994); Auewarakul, *et al.*, *Mol. Cell. Biol.* 14:8250-8258 (1994); Barbosa, *et al.*, *J. Virol.* 65:292-298 (1991); Kaur, *et al.*, *J. Gen. Virol.*
10 70:1261-1266 (1989); Schlegel, *et al.*, *EMBO J.*, 7:3181-3187 (1988)]. The constitutive expression of the E6/E7 proteins appears to be necessary to maintain the transformed condition of HPV-positive tumors.

Despite the capacity of some HPV strains to induce neoplastic phenotypes *in vivo* and *in vitro*, still other HPV types cause
15 benign genital warts such as condylomata acuminata and are only rarely associated with malignant tumors [Ikenberg. In Gross, *et al.*, (eds.) GENITAL PAPILLOMAVIRUS INFECTIONS. Springer Verlag: Berlin, pp., 87-112]. Low risk strains of this type include, for example, HPV 6 and 11.

Most often, genital papilloma viruses are transmitted between
20 humans during intercourse which in many instances leads to persistent infection in the anogenital mucous membrane. While this observation suggests that either the primary infection induces an inadequate immune response or that the virus has developed the ability to avoid immune surveillance, other observations suggest that the immune system is active
25 during primary manifestation as well as during malignant progression of papilloma virus infections [Altmann *et al.* in VIRUSES AND CANCER, Minson *et al.*, (eds.) Cambridge University Press, (1994) pp. 71-80].

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For example, the clinical manifestation of primary infection by rabbit and bovine papilloma virus can be prevented by vaccination with
30 wart extracts or viral structural proteins [Altmann, *et al.*, *supra*; Campo,

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Lutz Gissman and Martin Mueller) I hereby certify that this paper is being
Application Serial No. 10/042,526) deposited with the United States Postal
Filed: January 8, 2002) Service as First Class mail, postage
For: Papilloma Virus Capsomere) prepaid, in an envelope addressed to:
Vaccine Formulations and Methods of) Commissioner for Patents, Washington,
Use) D.C. 20231, on April 15, 2002.
Group Art Unit: 1648)
Examiner: TBD)
Eric M. Brusca

**APPLICANTS' RESPONSE TO NOTICE TO FILE MISSING PARTS OF
NONPROVISIONAL APPLICATION**

Commissioner for Patents
Washington, DC 20231

Sir:

In a Notice to File Missing Parts mailed February 2, 2002, various items were found to be missing in the above-identified application.

REMARKS

In order to avoid abandonment of the instant application, the Applicants file herewith a check covering the statutory basic filing fee and a late filing fee of \$130 as set forth in 37 CFR 1.16(l).

Additionally, a substitute specification in compliance with 37 CFR 1.52 is submitted herewith. The Applicants would like to point out that pages 1 and 2 of the specification have been corrected.

A copy of the Sequence Listing in computer readable form and corresponding statement that the content of the Sequence Listing information recorded in computer readable form is identical to the written Sequence Listing has been previously filed in the related application U.S.S.N 09/632,286. Thus, the Applicants submit that in accordance with 37 CFR 1.821(e), the sequence listing in the instant application is identical to that of U.S.S.N 09/632,286. The Applicants request that the computer-readable sequence listing in U.S.S.N 09/632,286 be entered in the present application.

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Parent SN
09/632,286 does
not have a
valid CRF.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Lutz Gissman and Martin
Mueller

Application Serial No. 10/042,526


Filed: January 8, 2002

For: Papilloma Virus Capsomere
Vaccine Formulations and Methods of
Use

Group Art Unit: 1648

Examiner: TBD

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